Phospholipase C-β (PLC-β) catalyzes the hydrolysis of the plasma membrane (PM) lipid phosphatidylinositol-(4,5)-bisphosphate ($PI(4,5)P_2$), thereby reducing the PM concentration of its precursor PI(4)P. A recent report proposed independent $PI(4,5)P_2$ and PI(4)P PM pools with essential physiological functions, but it remains unknown whether PLC-β targets to these pools differentially.

Here, we investigated the time course and specificity of PLC-β-dependent phospholipid depletion in living CHO cells using genetically-encoded phospholipid sensors, total internal reflection fluorescence microscopy and whole cell patch clamp. PLC-β mediated $PI(4,5)P_2$ hydrolysis was detected reliably using KCNQ (Kv7)-mediated $K^+$ currents, the $PI(4,5)P_2$ reporters $PLC\delta_1$-PH and Epsin ENTH, but not with the C-terminus of Tubby protein. Analogous experiments showed that activation of PLC-β did not reduce the concentration of PI(4) detected with the PI(4)P-specific reporter Osbp-PH. When PLC-β3 was heterologously overexpressed, Tubby-Cterm and Osbp-PH reported on the PLC-β-induced changes of $PI(4,5)P_2$ and PI(4)P, respectively.

In summary, we present detailed real-time analysis of PLC-β activity in living cells. Our findings indicate that phospholipid sensors may detect different phospholipid pools that are accessible to PLC-β differentially. Hence, this work supports the presence of functional phospholipid pools in living cells.

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**3149-Pos Board B304**

**Regulation of CAMP Compartmentation by Membrane Microdomains**

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The role of different membrane domains in the compartmentation of cAMP signaling was investigated using the FRET-based biosensor Epac2-camps. The MyrPalm sequence from Lyn kinase and the CAAX sequence from Rho GTPase were used to target this probe to lipid raft (Epac2-MyrPalm) or non-lipid raft (Epac2-CAAX) domains, respectively. Confocal imaging established that both probes were targeted to the plasma membrane in HEK293 cells. FRAP analysis demonstrated that depletion of membrane cholesterol altered both the recovery half-time and the mobile fraction of Epac2-MyrPalm but not Epac2-CAAX, confirming that each probe was targeted to the correct microdomain. FRET responses of these probes were then used to monitor relative changes in cAMP activity associated with lipid raft and non-raft domains. The results demonstrated that basal cAMP activity is significantly higher in non-raft domains. This was supported by the fact that the maximal increase in cAMP over baseline following agonist stimulation was significantly smaller for Epac2-CAAX than it was for Epac2-MyrPalm, consistent with the idea that the probe was partially activated by a higher basal level of cAMP associated with non-lipid raft domains. In addition, inhibition of adenylyl cyclase activity with MDL 12330A reduced basal cAMP activity detected by Epac2-CAAX but not Epac2-MyrPalm. Responses detected by Epac2-CAAX were also more sensitive to direct activation of adenylyl cyclase by forskolin, but less sensitive to inhibition of type 4 phosphodiesterase activity by rolipram. These results indicate that there are diffusionally-restricted pools of cAMP associated with different membrane microdomains under basal conditions. The higher basal cAMP activity associated with non-lipid raft domains can be explained by differences in basal adenylyl cyclase and phosphodiesterase activity.

**3150-Pos Board B305**

**Integrating High-Resolution Bioimaging Techniques to Unravel How Membrane Lipids Influence Nanoscale Organization and Lateral Mobility of Adhesion Receptors**

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A central, but still unresolved question regarding the function of integrins is how these adhesion receptors regulate both their conformation and dynamic nanoscale organization on the membrane to generate adhesion-competent microclusters upon ligand binding. By superresolution nanoscopy, we recently showed that in quiescent monocytes, LFA-1 preorganizes in ligand-independent nanoclusters proximal to nanoscale raft components (1,2). Furthermore, to dissect the relationship between conformational state, lateral mobility, and microclustering we exploited the high spatial (nanometer) accuracy and temporal resolution of single dye tracking and found that LFA-1 nanoclusters are primarily mobile on the cell surface with a small (ca. 5%) subset of conformational-active LFA-1 nanoclusters preanchored to the cytoskeleton (3). Lateral mobility resulted crucial for the formation of microclusters upon ligand binding and for stable adhesion under shear flow. Ongoing investigation in our laboratory points towards the importance of a specific lipid composition of the membrane nano-environment in modulating LFA-1 biophysical properties which eventually regulate the onset of leukocyte adhesion. Since several (patho)physiological stimuli can alter either temporarily or permanently the plasma membrane lipid composition, our studies offer a novel framework to understand integrin regulation via the lipid nanoenvironment.

(1) Van Zanten et al PNAS 2009.
(2) Van Zanten et al PNAS 2010.
(3) Bakker et al PNAS 2012.

**3151-Pos Board B306**

**Visualizing Ghrelin Receptor through Genetically Encoded Labeling for Monitoring the Single-Molecule Conformational Dynamics**

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The ghrelin receptor (GhR) is a class A G protein-coupled receptor (GPCR) involved in the entero-endocrine signaling systems that regulates food intake and energy homeostasis. The GhR is noted for its unusually high basal constitutively activity. GhR is a potential drug target for ''diabesity'' syndromes, and the interaction between GhR and its endogenous peptide ligand, ghrelin, has been intensively studied. However, there is only a limited understanding of GhR pharmacology and its molecular mechanism of signal transduction. Using well-established amber codon suppression technology and state-of-the-art single-molecule techniques, we are developing tools to monitor directly differential conformational dynamics of GhR in the presence and absence of its binding partners, including ligands, G proteins, or other GPCRs. For example, we are preparing single-site and double-site fluorescently labeled GhR and a series of labeled ghrelin analogues. These engineered receptors can be studied in cell-based systems or reconstituted in NABBs (Nanoscale Apolipoprotein Bound Bilayers) after purification. These types of approaches will enable us to better understand the complexity of GhR signaling in the neuro-endocrine system, providing insights to design specific drugs for targeting fine-tuned signal pathways involved in metabolic disorders like obesity and diabetes.

**3152-Pos Board B307**

**TGF-Beta and Bmp Receptors: Distinct Modes of Oligomeric Interactions and Implications for Signaling**

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Transforming growth factor-β (TGF-β) and bone morphogenetic protein (BMP) signal *via* two Ser-Thr kinase receptors, type I type II (TβRI and II, BMPRI and II for the TGF-β and BMP receptors, respectively), which appear at the cell surface as monomers, homomeric and heteromeric complexes. their extracellular domains (EDs) complexed with ligands were shown to form heterotetramers. However, the interaction dynamics among the full-length receptors in live cell membranes, the domains involved, and the potential roles of receptor homodimerization were largely unexplored. Using patch/FRAP and computerized immunofluorescence co-patching of epitope-tagged receptors [wild-type (wt) or mutants] in live cells, we show that the oligomerizaiton dynamics are distinctly different for the two receptor systems. For TGF-β receptors, we find clear differences between TβRII and TβRI oligomeric interactions: (1) the homodimerization of TβRII, but not TβRI, depends on a cytoplasmic juxtamembrane region; and (2) TβRI/TβRII hetero-oligomerization depends on the cytoplasmic domain of TβRI and on a C-terminal region of TβRII, distinct from the region involved in TβRII homodimerization. TGF-β1 binding mildly elevated TβRII homodimerization, and strongly enhanced TβRI/TβRII heteromeric complex formation. Notably, both homomeric and heteromeric TGF-β receptor complexes were stable on the patch/FRAP timescale (minutes).

In contrast, the BMP receptors display stable interactions on the same timescale only for homomeric complexes, while the heterocomplexes are transient. Interestingly, the BMPR heterocmplexes appear to form at the expense of homodimers, and stabilization of BMPRII/BMPRIb heteromeric (but not homomeric) complexes by IgG binding elevates phospho-Smad formation both without and with BMP-2. Based on these findings, we propose two mechanisms that can suppress the tendency of preformed BMP receptor hetero-oligomers to signal without ligand.